

## Nutrient Composition and Protein Quality of Four Species of the *Curcubitaceae* Family

Matilda Steiner-Asiedu, Peter Nuro-Ameyaw, Isaac Agbemafle, Sarah H. Hammond and  
Kweku Tano-Debrah

Department of Nutrition and Food Science, University of Ghana, P.O. Box LG 134, Legon, Accra, Ghana

**Abstract:** The kernels of most *Curcubitaceae* species have tremendous food value in Africa where protein-energy malnutrition is high but they are not widely used in food preparations. This study assessed the nutritional composition and protein quality of four species of *Curcubitaceae* seeds using laboratory rats. Moisture, ash, fibre, minerals and protein of the *Curcubitaceae* seeds were determined. Rats were randomly assigned to seven groups of five and were fed for four weeks on the following diets: the four species of the *Curcubitaceae* seed meal and control diets of Soya Bean Meal (SBM), protein free diet and normal rat chow respectively. Their urine and faeces were collected for analysis and feed intakes determined. After four weeks feeding, the rats were weighed, lyophilized and protein utilization determined. The moisture, crude protein, fat and ash content of the seeds were 5.44-6.66, 30-36, 44-58 and 3.18-4.90%, respectively. Crude fibre was less than 2.5%. The *Curcubitaceae* seeds contained high amounts of: Zn (5.0-7.1 mg/100 g), Cu (1.4-7.9 mg/100 g) and Fe (5.6-8.5 mg/100 g). All the seeds had good protein quality as judged by the PER (0.75-1.36) and (NPU) (46.10-69.10). The four *Curcubitaceae* seeds have good protein quality, fat and minerals which may help reduce under nutrition. These are important food resources and thus their consumption should be promoted.

**Keywords:** *Curcubitaceae* seeds, mineral, net protein utilization, protein efficiency ratio, protein quality

### INTRODUCTION

Protein Energy Malnutrition (PEM) is by far the most ruinous form of malnutrition, with children being the most visible casualties (WHO, 2010). Hence the search for alternative protein food source, especially for developing countries, is of utmost importance, particularly because of the high cost of animal protein sources and the high levels of poverty (Fasuyi, 2007). Nonetheless, most plant source foods have received much less attention in the scientific arena (Vodouhé and Capo-Chichi, 1998). This has restricted their cultivation to certain areas while other essential ones are still wild (Mnzava *et al.*, 1999); most of which contain proteins and other nutritionally important components that could be harnessed as alternatives for human diet (Achigan-Dako *et al.*, 2008; Mabalaha *et al.*, 2007). Hence a persuasive way to draw attention to local crops is to create awareness of their nutrient composition and protein quality for reducing PEM.

The discovery of protein and its effect on body metabolism (Cox and Nelson, 2001; Berg *et al.*, 2002), the higher commercial value placed on the protein product due to its necessity (Carpenter, 2003a, b and c) and the associated role it plays in preventing and/or solving malnutrition especially Protein Energy Malnutrition (WHO, 2010; Wardlaw, 2000) has for a long time triggered research into alternative sources and the various properties it possesses. Food has been

enriched both consciously and unconsciously to raise the protein status of protein deficient individuals via the use of scientifically approved methods especially relying on the complementarily of proteins from different sources, or the reliance on good traditionally adopted food preparation methods probably not with the real intention of improving the nutritional profile of the foods but to enhance the taste of food through harnessing available food stuffs (Ponka *et al.*, 2005).

Soybean, Peanuts, cowpea and different *Curcubitaceae* seeds have been used in different ways to ameliorate PEM. Some studies have also investigated the nutritional value, antinutrient constituents as well as the effect of processing on the anti-nutrient quantities of *Curcubitaceae* seeds in many part of West Africa (Fokou *et al.*, 2004; Onyeike and Acheru, 2002; Badifu, 2001). The seeds of most species of *Curcubitaceae* have tremendous food value in Africa (Badifu and Ogunsua, 1991), but information on biological utilization of its nutrients is scanty. Also, there are limited studies on the use of laboratory animals to clearly determine the bioavailability of the nutrients in the processed kernels of the different varieties of the *Curcubitaceae*. In Ghana four species of the *Curcubitaceae* namely: *Cucumeropsis mannii*, *Citrullus lanatus* (Thumb) Matsum. and Nakai var. *citroides*, *Cucumis melo* linn and *Cucurbita pepo* linn seeds are available but utilization is very low. To promote

utilization and consumption, particularly in complementary and/or supplementary feeding to help reduce the high rates of growth faltering in the country. It is important to know the nutrient profile as well as the biological utilization of the seeds in order to understand its fullest nutritional benefits. This study therefore determined the proximate composition, mineral content and the protein quality of seeds using laboratory rats.

## MATERIALS AND METHODS

**Sample and sample preparation:** Matured, sun-dried seed samples were obtained from three regions of Ghana where the seeds are popularly cultivated. *Cucumeropsis mannii*, *Citrullus lanatus* (Thumb.) Matsum. and Nakai var. *Citroides* and Soy beans were obtained from Northern region; *Cucumis melo* linn, obtained from Brong-Ahafo and *Cucurbita pepo* linn obtained from Brong-Ahafo and Western regions. The seeds were transported in polyethylene bags to the laboratory and authenticated at the Department of Botany, University of Ghana, Legon. The samples were dehulled manually and winnowed to remove all debris. The kernels obtained were ground in an electric grinder to fine powder, placed in air-tight containers and stored in the desiccator prior to further analysis.

**Proximate analyses:** The chemical composition of the *Cucurbitaceae* seeds were determined using standard methods after AOAC (1990). For moisture, Two grams of each of the samples was weighed and dried to constant weight at a temperature of 105°C to obtain the moisture content. Protein content was assessed using 0.2 g of samples which were digested using ammonium salts with concentrated H<sub>2</sub>SO<sub>4</sub> at a high temperature. The mixture was cooled and 50% NaOH added. The resulting ammonia was distilled into a known volume of standard boric acid with indicator. The distillate was then titrated with 0.1N HCl. The calculated amount of nitrogen obtained was multiplied by 6.25 to give the quantity of protein present in each of the samples.

Fat content was determined by the Soxhlet method as described by the American Oil Chemists' Society (1997) Crude fiber was calculated as the loss on ignition of dried residue remaining after digestion of sample with 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% NaOH solutions. The ash content was calculated as ash formed per the amount of sample used after 2 g of the samples were weighed into crucibles and heated to constant weight at a temperature of 550°C.

**Determination of minerals:** About 0.1 g of each of the prepared *Cucurbitaceae* seeds samples were wet digested with nitric acid and perchloric acid; the filtrates obtained were analyzed for Fe, Zn, Cu, Ca, Mn and Mg using the atomic absorption spectrophotometer (AOAC, 1990).

**Biological utilization of proteins:** To evaluate the protein quality of the seeds in terms of their biological utilization, animal modules were employed. To do this, the seeds were prepared and diets formulated as required by the nutrients needs of rats. Soy beans were used for control diet. The Net Protein Utilisation (NPU) and Protein Efficiency Ratio (PER) were obtained.

**Seed cake preparation:** Samples were roasted separately in oven at 100°C for 20 min, cooled and ground into powder with a laboratory mill (Size 8 inch laboratory mill, Christy and Norris limited, Chelmsford, England) and then passed through 1.0 mm sieve. Distilled water was mixed with the powder and filtered through 500 microns Impact Laboratory test sieve. The filtrates were boiled at 98°C for 20 min to further reduce the anti-nutrients in the anticipated seed meal to be prepared (Badifu, 2001). They were allowed to cool, freeze dried and defatted with the Soxhlet method of fat extraction using n-hexane as the extracting solvent (AOAC, 1990). The defatted samples were dried in an air oven at 60°C for 24 h to remove all traces of solvent. The seed cakes prepared which served as the protein source for the seed meal preparations were packed in air-tight plastic containers and refrigerated at 4°C. It is important to note that half the quantity of *C. mannii* was not roasted but taken through the subsequent processes as the other samples because it is not roasted when it is use in the preparation of stews such as palaver sauce.

**Formulation of animal meal:** The diets were prepared based on the formulation of the AOAC (1984) as shown in Table 1. All experimental diets were hand-mixed, with initial minute components of the ingredients to ensure uniformity and proper blending (Fasuyi, 2007). A separate Nitrogen free diet was made without any nitrogen source as an ingredient. Soy bean meal was prepared with the same procedure and served as the control diet. The protein content of the diet was 10%. The protein concentrated seed cakes of defatted soy bean as well as the *Cucurbitaceae* varieties to be evaluated were added to corn starch (BDH Limited Poole England) to give approximately 10% crude protein on a dry matter basis. Proximate composition of the diets used in the study was performed to ascertain and authenticate the proximate constituents of the diet. They were thereafter put into different well-sealed plastic containers, labelled and stored at 4°C prior to use.

**Experimental animals:** Forty clinically healthy weanling male albino rats (Sprague-Dawley), 24±2 days old were obtained from the clinical rat colony of the Centre for Scientific Research into Plant Medicine, Mampong-Akuapem, Ghana. The rats were randomly divided into 7 groups of 5 rats each on the basis of

Table 1: Composition of animal meal

| Ingredients                      | Concentration (g/Kg) |                   |                        |                            |                     |                          |                     |
|----------------------------------|----------------------|-------------------|------------------------|----------------------------|---------------------|--------------------------|---------------------|
|                                  | Soybean (control)    | Protein free diet | <i>C. mannii</i> (raw) | <i>C. mannii</i> (roasted) | <i>C. pepo</i> linn | <i>Cucumis melo</i> linn | * <i>C. lanatus</i> |
| Corn starch                      | 549.0                | 760               | 603.0                  | 603.0                      | 608.6               | 614.6                    | 611.6               |
| Defatted sample*                 | 211.0                | 0.0               | 157.0                  | 157.0                      | 151.4               | 145.8                    | 148.4               |
| Soybean oil                      | 80.0                 | 80.0              | 80.0                   | 80.0                       | 80.0                | 80.0                     | 80.0                |
| Fibre content of defatted sample | 8.0                  | 0.0               | 5.2                    | 5.2                        | 4.7                 | 4.3                      | 4.5                 |
| Cellulose                        | 2.0                  | 10.0              | 4.8                    | 4.8                        | 5.3                 | 5.7                      | 5.5                 |
| Sucrose                          | 100.0                | 100               | 100.0                  | 100.0                      | 100.0               | 100.0                    | 100.0               |
| Ash content of defatted sample   | 13.8                 | 0.0               | 10.5                   | 10.5                       | 13.0                | 9.9                      | 10.1                |
| Mineral mix <sup>a</sup>         | 26.2                 | 40.0              | 29.5                   | 29.5                       | 27.0                | 30.1                     | 29.9                |
| Total min. content               | 40.0                 | 40.0              | 40.0                   | 40.0                       | 40.0                | 40.0                     | 40.0                |
| Vitamin mix <sup>b</sup>         | 10.0                 | 10.0              | 10.0                   | 10.0                       | 10.0                | 10.0                     | 10.0                |

\*: To provide 10% protein level; <sup>a</sup>: Mineral mix (g/Kg diet), CoCl<sub>2</sub>.6H<sub>2</sub>O (0.001), CuSO<sub>4</sub>.5H<sub>2</sub>O (0.07), MnSO<sub>4</sub> (0.178), KI (0.032), KH<sub>2</sub>PO<sub>4</sub> (15.559), CaSO<sub>4</sub> (15.25), NaCl (5.573), ZnCO<sub>3</sub> (1.6), FeSO<sub>4</sub>.7H<sub>2</sub>O (1.078), MgSO<sub>4</sub>.7H<sub>2</sub>O (2.292); <sup>b</sup>: Vitamin mix (per Kg of diet): Thiamin hydrochloride = 6 mg, Pyridoxine hydrochloride = 7 mg; nicotinic acid = 30 mg; folic acid = 2 mg; calcium pantothenate = 16 mg; biotin = 0.2 mg; cyanocobalamin = 0.01 mg; retinol palmitate = 4000 IU; cholecalciferol = 1000 IU; α-tocopherol acetate = 50 IU; menadione = 0.05 mg; chlorine chloride = 2 g

initial weight and litter origin. They were housed individually in plastic metabolic cages to facilitate separate faecal and urinary collection in a room maintained at 18-26°C with a 12 h light/dark cycle and 40-70% relative humidity. They were fed commercial laboratory rat chow diet *Ad libitum* for a 4-day acclimatization period before they were placed on their respective test diets for 4 weeks.

**Net Protein Utilization (NPU):** After feeding for four weeks, the rats were humanely sacrificed with chloroform. The carcasses were frozen, lyophilized (Rasco, 2001), milled and their nitrogen contents assessed using the Kjeldahl method (AOAC, 1990). The quantity of nitrogen in the feed consumed during the entire 28-day period were also computed and used in the calculation of NPU:

$$NPU = \frac{\text{Body N of test group} - \text{body N of nonprotein group} + \text{N consumed by nonprotein group}}{\text{N consumed by test group}} \times 100$$

**Protein Efficiency Ratio (PER):** The rats were weighed every 7<sup>th</sup> day before and after each feeding episode until the end of the study. The PER was calculated based on weight gained by test group in grams divided by the total amount of protein consumed per each rat. Total protein was calculated based upon measurement of total nitrogen in the diet.

**Statistical analysis:** The data were represented as mean ± standard deviation of three experimental determinations. Between sample and within sample variations were evaluated using ANOVA and Turkey's test for multiple comparison was used to show where the differences are significant. Significance was set at p<0.05.

## RESULTS AND DISCUSSION

The carbohydrates content in the *Cucurbitaceae* seeds studied were lower than the other macronutrients in the seeds. The seeds of *C. melo* linn, *C. pepo* linn and *C. lanatus* exhibited similarities in their carbohydrate contents which ranged from 3.11±0.74-4.28±1.64% as shown in Table 2. This suggests that the seeds may have some resemblance in their genetic composition that is not affected by changes in climatic conditions (Achu *et al.*, 2005). The *C. mannii* was observed to be an ideal source of carbohydrate among the four *Cucurbitaceae* seeds species studied. The carbohydrate of *C. mannii* was found to be similar to the 7.11% found by Achu *et al.* (2005). On the contrary, the carbohydrates content of the *C. mannii* was lower than the 12.55% for the same variety that Abiodun and Odeleke (2010) reported. This implies that the *Cucurbitaceae* seeds studied may not serve as a good source of carbohydrates.

The lipid extracted from *C. mannii*, *C. lanatus* and *C. melo* linn, were pale yellow in colour while those from *C. pepo* linn seeds were green, similar to what Achu *et al.* (2005) detected. They were all fluid at room temperature. This suggests that they may have higher quantities of unsaturated fatty acids (Glew *et al.*, 2006; Giwa *et al.*, 2010; Oluba *et al.*, 2008; Uruakpa and Aluko, 2004). Glew *et al.* (2006) reported that the fatty acid: oleic, linoleic and stearic composition in *Cucurbita* sp., were: 45.4, 31.0 and 7.8%, respectively. Achu *et al.* (2008) also reported similar results when they analyzed the fatty acid composition of the oil of five different *Cucurbitaceae* species. These finding suggests that the *Cucurbitaceae* varieties investigated could provide the fatty acids for the synthesis of other fatty acid derivatives which could be harnessed for the growth and development of consumers especially children (Williams, 2005; Wardlaw, 2000; Anthony, 2000).

Table 2: Nutritive content of dry decorticated seeds of four *Cucurbitaceae* species

| Nutrient         | Sample (%±S.D.)         |                         |                         |                         |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                  | <i>C. manni</i>         | <i>C. melo</i> linn     | <i>C. pepo</i> linn     | <i>C. lanatus</i>       |
| Protein          | 36.00±0.24 <sup>a</sup> | 35.00±0.39 <sup>a</sup> | 36.00±0.94 <sup>a</sup> | 30.00±0.39 <sup>b</sup> |
| Fats             | 44.00±0.58 <sup>d</sup> | 50.00±0.37 <sup>b</sup> | 46.00±0.57 <sup>c</sup> | 56.00±0.29 <sup>a</sup> |
| Moisture         | 6.64±0.09 <sup>a</sup>  | 6.34±0.17 <sup>a</sup>  | 6.66±0.25 <sup>a</sup>  | 5.44±0.14 <sup>b</sup>  |
| <sup>1</sup> CHO | 7.65±0.29 <sup>a</sup>  | 3.11±0.74 <sup>b</sup>  | 4.28±1.64 <sup>b</sup>  | 4.13±0.71 <sup>b</sup>  |
| Fibre            | 2.00±0.31 <sup>a</sup>  | 2.00±0.33 <sup>a</sup>  | 2.40±0.48 <sup>a</sup>  | 1.90±0.39 <sup>a</sup>  |
| Ash              | 3.91±0.03 <sup>a</sup>  | 3.56±0.06 <sup>b</sup>  | 4.90±0.08 <sup>c</sup>  | 3.18±0.06 <sup>d</sup>  |

*C. lanatus* is *Citrullus lanatus* (Thumb.) Matsum. and Nakai var. *citroides*; <sup>1</sup>: Carbohydrate; <sup>a-c</sup>: Values within a row with the same superscript means that there are no significant differences but those with different superscript show significant differences (p<0.05) by the Tukey's multiple comparison test; S.D.: Standard deviation

The fat (lipid) contents of *C. manni*, *C. melo* linn, *C. Pepo* linn and *C. lanatus* were 44.00±0.58, 50.00±0.37, 46.00±0.57%, 56.00±0.29%, respectively (Table 2). The lipid content of *C. lanatus* was similar to the 56.67±4.90% obtained by Loukou *et al.* (2007). *C. manni* lipid content obtained was also comparable to what was obtained by Loukou *et al.* (2007) and Anhwange *et al.* (2010). It was however, higher than the 42.29% obtained by Abiodun and Adeleke (2010). The quantity of lipid obtained from the *C. pepo* linn assessed was also higher than that obtained by Nyam *et al.* (2009). This may be due to the method used in the sample preparation stage. It was not stated by Nyam *et al.* (2009) whether they dehulled the seeds used for their lipid quantification. If they did not dehull the seeds prior to their use, the testa of the seeds might have absorbed some of the lipid that would have been measured. The higher lipid content we obtained might also be attributed to the genetic makeup or environmental conditions that might have favored higher lipid synthesis by the seeds (Achu *et al.*, 2005).

More than 40% of the content of the seeds of the studied *Cucurbitaceae* species being lipid is indicative of their prolific lipid content. The lipid content shows that they, (especially *C. lanatus*) could be alternative to soy bean which has been reported by USDA (2010) to have 20%. The quantities of lipid in the seeds provides a vital evidence that they could also be considered for use in the formulation of lipid based nutrient supplementation products particularly due to their added high protein content. The *Cucurbitaceae* seeds investigated have high economic potential as commercial lipid seeds. This advantage could be harnessed to improve the financial well-being of impoverished rural people who are mostly the growers of these *Cucurbitaceae* seeds.

The quantity and quality of protein in the seeds were assessed in order to ascertain the amount of this nutrient these plant species could contribute to the diet of consumers as well as their prolificacy as a source of protein in the industrial and research setting. The protein contents of all the *Cucurbitaceae* varieties studied were above 30% indicating that they are rich sources of proteins. *C. manni*, *C. melo* linn, *C. pepo* linn and *C. lanatus* had protein content of 36.00±0.24, 35.00±0.39, 36.00±0.94 and 30.00±0.39%, respectively

(Table 2). The protein content of *C. lanatus* was comparable to what was obtained by Loukou *et al.* (2007) but lower than another variety of *C. lanatus* (watermelon) that El-Adawy and Taha (2001) investigated. The protein content of *C. pepo* linn was higher than that obtained by Nyam *et al.* (2009), but comparable to the result of El-Adawy and Taha (2001). It was however; lower than the result obtained by Younis *et al.* (2000). Likewise, the protein content of *C. melo* linn obtained in this study was found to be significantly (p<0.05) higher than the 29.55% obtained in *C. melo* var. *agrestis* (Loukou *et al.*, 2007). However that of the *C. manni* was similar to the 36.1%, obtained by Badifu and Ogunsua (1991), as well as the 34.64 to 41.18% in *C. manni* grown in the high savanna region of Cameroon, but lower than the 43.38 and 41.75% in the same species cultivated in the rain and swamp forests respectively grown in the same country (Achu *et al.*, 2005). This suggest that the variability observed could be attributed to intra-species and interspecies differences or seed drying procedures (Bankole *et al.*, 2005) as well as the different climatic conditions.

The protein content of all the *Cucurbitaceae* varieties studied were found to be higher than those of other lipid seeds such as cashew nuts (22.8%), cottonseed (21.9%) and sesame (18.7%) (FAO, 1982). It is worth noting that *C. manni*, *C. melo* linn and *C. pepo* linn could provide consumers proteins in quantities comparable to the 36.49% of soy bean (USDA, 2010). About 55 g of the *Cucurbitaceae* seeds studied could provide the Recommended Dietary Intake (RDI) of 19 g needed by children at age 5 (Institute of Medicine, 2001). These species may therefore be able to provide enough proteins for the nutritionally vulnerable, especially children, pregnant and lactating mothers, as well as old people who need adequate proteins in their diet for proper growth, maintenance and repair of worn out tissues (Williams, 2005). It also suggests that the *Cucurbitaceae* varieties studied are capable of providing enough proteins that could be added to staples that are poor in proteins and/or some of the amino acids to provide a very good diet for children especially during complementary feeding (Asiedu *et al.*, 1994) to help solve the increasing demand of proteins for children especially in developing countries.

The fraction of the proteins consumed which is absorbed by the body cells (net protein) and used to

Table 3: Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) of the proteins in the seed cakes of four *Cucurbitaceae* species and soybean

| Feed type                               | PER±S.D.               | NPU±S.D.                   |
|---|------------------------|----------------------------|
| <i>C. melo</i> linn                     | 1.25±0.08 <sup>c</sup> | 54.40±1.60 <sup>a</sup>    |
| <sup>1</sup> <i>C. mannii</i> (raw)     | 1.17±0.07 <sup>c</sup> | 51.00±1.32 <sup>b, f</sup> |
| Soya bean                               | 1.87±0.04 <sup>a</sup> | 67.70±1.53 <sup>c</sup>    |
| <i>C. lanatus</i>                       | 0.75±0.03 <sup>d</sup> | 46.10±1.62 <sup>d</sup>    |
| <i>C. pepo</i> linn                     | 1.36±0.01 <sup>b</sup> | 61.00±1.64 <sup>e</sup>    |
| <sup>2</sup> <i>C. mannii</i> (roasted) | 1.25±0.06 <sup>c</sup> | 53.70±2.11 <sup>a, f</sup> |

<sup>a-f</sup>: Values within a column with the same superscript means that there are no significant differences but those with different superscript show significant differences (p<0.05) by the Tukey's multiple comparison test; S.D.: Standard deviation

synthesize new cells for growth and repair of worn out tissues is of immense benefit. Net Protein Utilization (NPU) is therefore used as a means of predicting the quality (biological value) of a protein (Rasco, 2001). The NPU for the *Cucurbitaceae* varieties studied ranged from 46.10±1.62-61.00±1.64 (Table 3). The NPU for soy bean (67.70±1.53) as shown in Table 3 was higher than all the *Cucurbitaceae* species investigated but *C. pepo* linn had the highest NPU among all the *Cucurbitaceae* varieties. The *C. pepo* linn could thus be regarded as the best protein source among the *Cucurbitaceae* species for providing much essential amino acids for the growth and maintenance of consumers especially vulnerable people like: children, pregnant women, the elderly and the sick (Williams, 2005; Wardlaw, 2000). The NPU for *C. melo* linn and *C. pepo* linn were higher than all the NPU for the various processed *C. vulgaris* schrad obtained by Oyenuga and Fetuga (1975). On the other hand, there was no significant difference between the NPU for *C. mannii* (raw) and *C. mannii* (roasted) and the 50.12 for Fried Defatted Meal (FDM) made from *C. vulgaris* schrad that Oyenuga and Fetuga (1975) found. The NPU for *C. lanatus* was lower than the Fried Defatted Meal (FDM) and the Raw Defatted Meal (RDM); but higher than the NPU for the Fried Undefined Meal (FUM) and the Raw Undefined Meal (RUM) from the same seed variety. This indicates that the pre-treatment method employed impacted significantly on the NPU of the studied seeds.

The quantity of tissue developed which provide the platform for growth out of the protein consumed as proposed by Osborne *et al.* (1919) has been used as a method for assessing protein quality. Protein Efficiency Ratio (PER) of the *Cucurbitaceae* seeds varied from 1.36±0.01 for *C. pepo* linn to 0.75±0.03 for *C. Lanatus* (Table 3). The PER of 1.36 recorded for the *C. pepo* linn was higher than the 1.01 recorded by Zdunczyk *et al.* (1999) and Oyenuga and Fetuga (1975) for other varieties of *Cucurbitaceae* investigated. Some changes in processing the test diet or differences in the genetic makeup of the *C. pepo* from different parts of the world might have contributed to the changes in the PER (Achu *et al.*, 2005). The PERs for all the seeds obtained were however lower than the PER of the 10% content

of germinated fluted pumpkin (*Telfairia occidentalis* Hook) concentrates in cookies that Giami and Barber (2004) reported. The lowest PER of 0.75 obtained for the *C. lanatus* indicates that the proteins from other plant sources have to be added to augment the essential amino acids to be consumed. As Zdunczyk *et al.* (1999) did, the addition of soy bean to *C. pepo* at the ratio of 1:1 raised the PER of the product to 1.43 which was higher than the PER obtained for soy bean or *C. pepo* alone. The PER obtained for *C. lanatus* tallies with its level of coagulation observed during the processing of the various seed meal. It appeared to have the least level of coagulation. It may be having low levels of soluble proteins like albumins and globulins that were utilized by the rats used in the bioassay.

Similarity in PER was observed in the PERs of *C. mannii* both raw and roasted and *C. melo* linn. This indicates that these protein sources have similar protein characteristics that were utilized by the rats (Giami and Barber, 2004). They could therefore be harnessed interchangeably as a source of protein in any protein based diet. The absence of significant difference in the PER of the *C. mannii* (raw) and *C. mannii* (roasted) also suggests that boiling alone or roasting before boiling that most Ghanaians incorporate in the cooking of these *Cucurbitaceae* varieties do not produce any differences in the biological utilization of the proteins in them. According to Badifu (2001), roasting alone is capable of reducing 22-44% saponin and 24-54% total phenol contents of *Cucurbitaceae* kernels; whereas boiling can also appreciably reduce all anti-nutrients by about 40-81%, with phenols having the greatest reduction. Thus, the processing method involving roasting before boiling might have been expected to further reduce substantially the amounts of anti-nutrients to bring about an increase in the bioavailability of the proteins in the seed cake used. The roasting done before boiling might have been added by users to improve the organoleptic properties of the outcome-usually the soup. There was a lower PER value of the proteins of the seeds of the *Cucurbitaceae* varieties as compared to that of the soy bean (1.87±0.04) based on the method employed. It suggests that the essential amino acids and/or the bioavailability of the proteins of the soy bean may be superior to that of the seeds of the *Cucurbitaceae* varieties studied. However, different procedures like the inclusion of germination in the processing of seeds before formulation of the test diet could have had a positive influence on the PER (Giami and Barber, 2004; Asiedu *et al.*, 1994). Hence different methods of processing need to be investigated to determine the most appropriate method of making the proteins in the *Cucurbitaceae* seeds more bio-available.

The search for good iron sources is relevant due to the increasing levels of Iron deficiency anaemia in Africa. The iron content of *C. mannii*, *C. melo* linn, *C. Pepo* linn and *C. lanatus* were 7.10±0.00, 8.50±0.01,

Table 4: Mineral profile of the dry seeds of four *Cucurbitaceae* species

| Mineral | Sample (mg/100 g, dry weight) |                          |                          |                          |
|---------|-------------------------------|--------------------------|--------------------------|--------------------------|
|         | <i>C. mannii</i>              | <i>C. melo</i> linn      | <i>C. pepo</i> linn      | <i>C. lanatus</i>        |
| Fe      | 7.10±0.00 <sup>b</sup>        | 8.50±0.01 <sup>a</sup>   | 6.50±0.00 <sup>c</sup>   | 5.60±0.00 <sup>d</sup>   |
| Cu      | 1.40±0.36 <sup>a</sup>        | 1.60±0.58 <sup>a</sup>   | 7.90±0.41 <sup>b</sup>   | 1.50±1.90 <sup>a</sup>   |
| Mn      | 4.40±0.00 <sup>a</sup>        | 3.40±0.00 <sup>b</sup>   | 3.20±0.01 <sup>c</sup>   | 2.40±0.00 <sup>d</sup>   |
| Zn      | 5.00±0.07 <sup>d</sup>        | 5.80±0.03 <sup>b</sup>   | 7.10±0.04 <sup>a</sup>   | 5.10±0.02 <sup>c</sup>   |
| Mg      | 430.00±17.00 <sup>b</sup>     | 430.00±0.34 <sup>b</sup> | 530.00±0.35 <sup>a</sup> | 350.00±0.68 <sup>c</sup> |
| K       | 620.00±1.00 <sup>a</sup>      | 560.00±0.34 <sup>c</sup> | 610.00±1.00 <sup>b</sup> | 540.00±0.68 <sup>d</sup> |
| Ca      | 44.00±23.00 <sup>a</sup>      | 29.00±14.00 <sup>b</sup> | 31.00±3.90 <sup>b</sup>  | 34.00±2.60 <sup>b</sup>  |
| Na      | 9.30±0.06 <sup>b</sup>        | 14.00±0.02 <sup>a</sup>  | 8.50±0.06 <sup>d</sup>   | 8.70±0.03 <sup>c</sup>   |

<sup>a-d</sup>: Values within a row with the same superscript means that there are no significant differences but those with different superscript show significant differences (p<0.05) by the Tukey's multiple comparison test

6.50±0.000 and 5.60±0.00 mg/100g, respectively (Table 4). The iron content in *C. pepo* linn studied was found to be lower than the 10.9 mg/100 g as well as the 106 µg/g observed by El-Adawy and Taha (2001) and Glew *et al.* (2006) respectively. *C. mannii* was observed to contain lower iron content than the 117 ppm reported by Badifu and Ogunsua (1991). According to American Academy of Pediatrics (1993), the Recommended Daily Intake (RDI) for iron for infant between the ages 6 to 12 months of weight 9 kg is 7 mg. The result suggests that *C. mannii* and *C. melo* linn may be capable of providing more than 100% of the recommended daily iron intake if these seeds could constitute 100 g of the total food consumed per day. *C. pepo* linn and *C. lanatus* could contribute 93 and 80%, respectively. All the seeds with the exception of *C. lanatus* could provide all the daily iron needed by children between 1 and 3 years and weights between 11 and 14 kg. However, only *C. melo* linn could provide the RDI for children at their fifth year. *C. mannii*, *C. pepo* linn and *C. lanatus* may be capable of providing 89, 81 and 70% RDI, respectively of iron for children at age five.

Conversely, since the absorption of iron from plant food sources is low, there is the need to consume alongside these iron rich sources some iron absorption enhancers like vitamin C-a cheaper enhancer than meat to augment the absorption of the iron. The substantial iron content in the studied *Cucurbitaceae* species coupled with its high protein contents could go a long way in the synthesis of iron containing compounds like haemoglobin, myoglobin and cytochromes that play pivotal role in energy metabolism (Cox and Nelson, 2001). Increased consumption of these *Cucurbitaceae* as part of the meal could also increase work efficiency among men and women in their productive ages. This will increase their income levels that would also help raise their living condition which has been seen to have influence on the nutritional status of their families.

Abundance of physiological energy in children especially would also enhance their growth and development necessary in their critical stage of development due to the rapid rate of growth and differentiation of cells of the children. These *Cucurbitaceae* seed varieties could also be used to curtail iron deficiency anaemia among women of child

bearing age and during pregnancy; because of increased iron needed to supply the expanding blood volume of the mother, rapidly growing foetus and placenta (Adom *et al.*, 2010; Bowman and Russell, 2001). These rich iron sources could also help to reduce the prevalence of associated effects like low birth weight mainly in the form of intrauterine growth retardation, preterm delivery and foetal death partly due to iron deficiency anaemia in pregnant women especially in developing countries (Centres for Disease Control and Prevention, 1998).

Research into the benefits of zinc to plants and animals has received tremendous attention since 1926. The zinc content in *C. mannii*, *C. melo* linn, *C. pepo* linn and *C. lanatus* were 5.00±0.07, 5.80±0.03, 7.10±0.04 and 5.10±0.02 mg/100 g, respectively (Table 4). The zinc content in *C. pepo* linn studied was found to be lower than the 8.2 mg/100 g as well as the 113 µg/g reported by El-Adawy and Taha (2001) and Glew *et al.* (2006), respectively. All the seeds could also provide more than 100% of RDI for zinc for children at age 5 years (American Academy of Pediatrics, 1993; Institute of Medicine, 2001).

The studied *Cucurbitaceae* could be promising in the provision of zinc to consumers especially children because zinc functions in the synthesis of both deoxyribonucleic and ribonucleic acids that play vital roles in cellular growth and differentiation. Besides these nucleic acids have influence on tissues with rapid cell turnover, especially the immune system which requires rapid expansion of immune cells including natural killer cells and phagocytic polymorphonuclear leukocytes (Bowman and Russell, 2001). The intake of these good zinc sources could also enhance the growth, the sexual wellbeing, vision and the social comfort of the consumers. Among the pregnant, it could help prevent prolonged labor, intrauterine growth retardation, teratogenesis and foetal death. It could also help raise the zinc status of lactating mothers especially at their early weeks of postpartum which have been known to pose a significant threat to maternal zinc status (Krebs, 1998).

The copper content of the *Cucurbitaceae* species ranged from 1.40±0.36-7.90±0.41 mg/100 g as shown in Table 4. Copper content in *C. pepo* linn studied was found to be about four times higher than the 1.7 mg/100

g and 15.4 µg/g that El-Adawy and Taha (2001) as well as Glew *et al.* (2006) respectively observed. The higher percentages of copper especially in *C. pepo* linn seeds taking into consideration RDI for copper may be attributed to genetic differences among the various *Cucurbitaceae* varieties (Achu *et al.*, 2005). According to the recommendation of the Institute of Medicine (2001), the copper content in *C. mannii*, *C. melo* linn, *C. pepo* linn and *C. lanatus* could make available about: 636, 727, 3590 and 681%, respectively for children between 7 and 12 months; 412, 471, 2300 and 440%, respectively for children between 1 and 3 years; and 318, 364, 1795 and 341%, respectively for children between 4 and 8 years if 100 g of these *Cucurbitaceae* is consumed by a child and also serves as a sole provider of copper a day.

The essentiality of this transitional metal has been known for over 70 years. It is present in biological systems as either oxidized cupric ion (Cu<sup>2+</sup>) and the reduced cuprous (Cu<sup>+</sup>) ion (Williams, 2005). The homeostatic regulation of copper absorption and excretion (Bremner, 1998) as well as the impact of anti-nutrients on the absorption of this mineral (Badifu, 2001) could make the consumption of these copper rich seeds safe. The much higher content of copper in the *Cucurbitaceae* could be greatly harnessed by pregnant and lactating mothers who need between 1000 and 1300 µg/day (Institute of Medicine, 2001). Copper is essential component of many proteins including several metalloenzymes like cytochromes oxidase and lysyl oxidase that are pivotal in the oxidative metabolism in the electron transport chain and the cross linking of elastin respectively. The transport of iron could also be compromised if copper is deficient in the plasma, as about 90% of copper in the plasma is bound to ceruloplasmin an important complex in iron transport and availability (Bowman and Russell, 2001).

### CONCLUSION

The *Cucurbitaceae* seeds had low carbohydrate but high fat and protein content as compared to other plant sources. Crude fibre levels were less than 2.5% in all samples. The seeds contained high amounts of Zn, Cu and Fe. All the seeds had good protein quality comparable to soy bean as judged by the PER and NPU. The research findings indicate that these under-utilized *Cucurbitaceae* seeds available in Ghana could be used to add value to traditional foods to combat Protein Energy Malnutrition as well as zinc and iron deficiencies.

### LIMITATIONS

Even though the feed prepared were having the same energy contents, the quantity of food consumed by the rats fed the *Cucurbitaceae* seed meals were

lower than that consumed by those fed on the soy bean meal. Further research into the fatty acid and amino acid profiles of the *Cucurbitaceae* seeds should be investigated since it may impact on the palatability of the seeds.

### ACKNOWLEDGMENT

We, the authors, are grateful to the technicians of the Nutrition and Food Science Department, University of Ghana and Centre for Scientific Research into Plant Medicine, Mampong Akuapem, Ghana for their technical assistance.

### REFERENCES

- Abiodun, O.A. and R.O. Adeleke, 2010. Comparative studies on nutritional composition of four melon seeds varieties. Pak. J. Nutr., 9: 905-908.
- Achigan-Dako, E.G., R. Fagbemissi, T.A. Hermene, R.S. Vodouhe, O. Coulibaly and A. Ahanchede, 2008. Importance and practices of Egusi crops (*Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumeropsis mannii* Naudin and *Lagenaria siceraria* (Molina) Standl. cv. 'Aklamkpa') in sociolinguistic areas in Benin. Biotechnol. Agron. Soc., 12: 393-403.
- Achu, M.B., E. Fokou, C. Tchiégang, M. Fotso and F.M. Tchouanguep, 2005. Nutritive value of some Cucurbitaceae oilseeds from different regions in Cameroon. Afr. J. Biotechnol., 4: 1329-1334.
- Achu, M.B., E. Fokou, C. Tchiégang, M. Fotso and T.F. Mbiapo, 2008. Atherogenicity of *Cucumeropsis mannii* and *Cucumis sativus* oils from Cameroon. Afr. J. Food Sci., 2: 021-025.
- Adom, T., M. Steiner-Asiedu, E. Sakyi-Dawson and A.K. Anderson, 2010. Effect of fortification of maize with cowpea and iron on growth and anaemia status of children. Afr. J. Food Sci., 4: 136-142.
- American Academy of Pediatrics, 1993. Pediatrics Nutrition Handbook. 3rd Edn., USA, pp: 94-98.
- Anhwange, B.A., B.A. Ikyenge, D.T. Nyiatagher and J.T. Ageh, 2010. Chemical Analysis of *Citrullus lanatus* (Thunb.), *Cucumeropsis mannii* (Naud.) and *Telfairia occidentalis* (Hook F.) seeds oils. J. Appl. Sci. Res., 6: 265-268.
- Anthony, M.S., 2000. Soy and cardiovascular diseases: Cholesterol lowering and beyond. Proceeding of the 3rd International Symposium on the Role of Soy in Preventing and Treating Chronic Diseases, 130: 662S-663S.
- AOAC, 1984. Official Methods of Analysis. 14th Edn., Association of Official Analytical Chemists, Washington, DC.

- AOAC, 1990. Official Methods of Analysis. 13th Edn., Association of Official Analytical Chemists, Washington, DC., U.S.A., pp: 684.
- Asiedu, M., E. Lied and R. Nilsen, 1994. Protein utilization and in-vitro protein synthesis in young rats given gruels of sprouted white maize supplemented with graded amounts of dried fish. *Food Chem.*, 49: 299-303.
- Badifu, G.I., 2001. Effect of processing on proximate composition, antinutritional and toxic contents of kernels from *Cucurbitaceae* species grown in Nigeria. *J. Food Compos. Anal.*, 14: 153-161.
- Badifu, G.L.O. and A.O. Ogunsua, 1991. Chemical composition of kernels from some species of *Cucurbitaceae* grown in Nigeria. *Plant Food Hum. Nutr.*, 41: 35-44.
- Bankole, S.A., A. Osho, A.O. Joda and O.A. Enikuomelin, 2005. Effect of drying method on the quality and storability of 'egusi' melon seeds (*Colocynthis citrullus* L.). *Afr. J. Biotechnol.*, 4:799-803.
- Berg, J.M., J.L. Tymoczko and L. Stryer, 2002. *Biochemistry*. 5th Edn., WH Freeman and Co., New York, pp: 41-690.
- Bowman, B.A. and R.M. Russell, 2001. *Present Knowledge in Nutrition*. 8th Edn., ILSI Press, Washington, USA, pp: 273-384.
- Carpenter, K.J., 2003a. A short history of nutritional science: Part 2 (1885-1912). *J. Nutr.*, 133: 975-984.
- Carpenter, K.J., 2003b. A short history of nutritional science: Part 3 (1912-1944). *J. Nutr.*, 133: 3023-3032.
- Carpenter, K.J., 2003c. A short history of nutritional science: Part 4 (1945-1985). *J. Nutr.*, 133: 3331-3342.
- Centres for Disease Control and Prevention, 1998. Recommendations to prevent and control iron deficiency in the United States. *MMWR Recommendations and Reports*, 47(RR-3): 1-29.
- Cox, M.M. and D.L. Nelson, 2001. *Lehninger Principles of Biochemistry*. 4th Edn., Freeman Publishers, New York, pp: 2.
- El-Adawy, T.A. and K.M. Taha, 2001. Characteristics and composition of different seed oils and flours. *Food Chem.*, 74: 47-54.
- FAO, 1982. *Food Composition Table for the Near East. Nuts and Seeds*. FAO Food and Nutrition Paper, 26. pp: 85, ISBN: 92-5-101277-6.
- Fasuyi, A.O., 2007. Bio-nutritional evaluations of three tropical leaf vegetables (*Telfairia occidentalis*, *Amaranthus cruentus* and *Talinum triangulare*) as sole dietary protein sources in rat assay. *Food Chem.*, 103: 757-765.
- Fokou, B., M.B. Achu and T.M. Chaungues, 2004. Preliminary nutritional evaluation of five species of egusi seeds in Cameroon. *Afr. J. Food Agric. Nutr. Dev.*, 4: 8-14.
- Giambi, S.Y. and L.I. Barber, 2004. Utilization of protein concentrates from ungerminated and germinated fluted pumpkin (*Telfairia occidentalis* hook) seeds in cookie formulations. *J. Sci. Food Agr.*, 84: 1901-1907.
- Giwa, S., L.C. Abdullah and M.A. Nor, 2010. Investigating "Egusi" (*Citrullus Colocynthis* L.) seed oil as potential biodiesel feedstock. *Energies*, 3: 607-618.
- Glew, R.H., R.S. Glew, L.T. Chuang, Y.S. Huang, M. Millson, D. Constans and D.J. Vanderjagt, 2006. Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita spp*) and *Cyperus esculentus* nuts in the republic of Niger. *Plant Food Hum. Nutr.*, 61: 51-56.
- Institute of Medicine, 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*. National Academy Press, Washington, DC.
- Krebs, N.F., 1998. Zinc supplementation during lactation. *Am. J. Clin. Nutr.*, 68: 509-512.
- Loukou, A.L., D. Gnakri, Y. Dje, A.V. Kippre, M. Malice, J.P. Baudoin and I.A. Zoro Bi, 2007. Macronutrient composition of three cucurbit species cultivated for seed consumption in Cote d'Ivoire. *Afr. J. Biotechnol.*, 6: 529-533.
- Mabalaha, M.B., Y.C. Mitei and S.O. Yoboah, 2007. A comparative study of the properties of selected melon seeds oils as potential candidates for development into commercial edible vegetable oil. *J. Am. Oil Chem. Soc.*, 84: 31-34.
- Mnzava, N.M., J.A. Dearing, L. Guarino, J.A. Chweya and H. de Koeijer, 1999. *Bibliography of the Genetic Resources of Traditional African Vegetables. Neglected Leafy Green Vegetable Crops in Africa*. Vol. 2. International Plant Genetic Resources Institute, Rome, Italy.
- Nyam, K.L., C.P. Tan, O.M. Lai, K. Long and Y.B. Che Man, 2009. Physicochemical properties and bioactive compounds of selected seed oils. *LWT-Food Sci. Technol.*, 42: 1396-1403.
- Oluba, M.O., Y.R. Ogunlowo, G.C. Ojieh, K.E. Adebisi, G.O. Eidangbe and I.O. Isiosio, 2008. Physicochemical properties and fatty acid composition of *Citrullus lanatus* (Egusi melon) seed oil. *J. Biol. Sci.*, 8: 814-817.
- Onyeike, E.N. and G.N. Acheru, 2002. Chemical composition of selected Nigeria oil seeds and physicochemical properties of the oil extracts. *Food Chem.*, 77: 431-437.
- Osborne, T.B., L.B. Mendel and E.L. Ferry, 1919. A method of expressing numerically the growth-promoting value of proteins. *J. Biol. Chem.*, 37(2): 223.
- Oyenuga, V.A. and B.L. Fetuga, 1975. Some aspects of the biochemistry and nutritive value of the water melon seed (*Citrullus vulgaris*, schrad). *J. Sci. Food Agr.*, 26: 843-854.

- Ponka, R., E. Fokou, M. Fotso, M.B. Achu and F.M. Tchouanguep, 2005. Methods of preparation and the energy, protein and mineral values of three cameroonian dishes: "Corn chaff", "nnam owondo/ebobolo" and "nnam ngon/ebobolo". *Afr. J. Food Agric. Nutr. Dev.*, 5: 1-12.
- Rasco, B., 2001. Biochemical Compositional Analysis of Proteins. *Current Protocols in Food Analytical Chemistry*. John Wiley and Sons Inc., Hoboken, NJ, USA, pp: B2.1.1-B2.1.15.
- Uruakpa, F.O. and R.E. Aluko, 2004. Heat-induced gelation of whole egusi (*Colocynthis citrullus* L.) seeds. *Food Chem.*, 87: 349-354.
- USDA, 2010. Agricultural Research Service. National Nutrient Database for Standard Reference, Release 23.
- Vodouhé, S.R. and L. Capo-Chichi, 1998. Egusi: High Protein Crop with Multiple Uses but Neglected and Under-Utilised. *Bulletin-CIEPCA/West Africa Cover Crops*. Cotonou, Republic of Benin, pp: 6.
- Wardlaw, G.M., 2000. *Contemporary Nutrition*. 4th Edn., McGraw Hill, New York, pp: 176-197.
- WHO (World Health Organization), 2010. *World Health Statistics*. WHO Library Cataloguing-in-Publication Data, World Health Organization, pp: 12.
- Williams, M.H., 2005. *Nutrition for Health, Fitness and Sports*. 7th Edn., McGraw Hill, New York, pp: 208-240.
- Younis, Y.M., S. Ghirmay and S.S. Al-Shihry, 2000. African *Cucurbita pepo* L.: Properties of seed and variability in fatty acid composition of seed oil. *Phytochemistry*, 54: 71-75.
- Zdunczyk, Z., D. Minakowski, S. Frejnagel and M. Flis, 1999. Comparative study of the chemical composition and nutritional value of pumpkin seed cake, soybean meal and casein. *Nahrung*, 43: 392-5.